RELATIONSHIP BETWEEN BIOLOGICAL PROPERTIES OF THE VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS AND SIZES OF THE VIRIONS

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Relationship between biological properties of the Venezuelan equine encephalomyelitis virus and sizes of the virions

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Abstract: In natural genotypes of the Venezuelan equine encephalomyelitis virus there is a correlation between the size of the virion and the size of the plaques (correlation coefficient r = -0.95). The thermostability of the virus, its pathogenicity for white mice, and its capacity for multiplication at 40° C do not depend upon the size of the virus particles. Clones of the virus with relatively small and middle-sized virions are characterized by the capacity for autointerference and by sensitivity to the inhibiting effect of agar polysaccharides. In clones with large virions, these genetic characteristics may show an opposite phenotypic manifestation. These regularities do not hold true for temperature mutants which have lost pathogenicity for white mice and have lost the capacity for multiplication at 40° C. The size of the virions undergoes changes in the course of mutations induced by 5-fluorouracil. The observed increase in the size of virions in mutants is accompanied by loss of the capacity of the virus to produce large plaques.

We have reported earlier that the size of virions of the Venezuelan equine encephalomyelitis (VEE) virus belongs to the category of genetic characteristics. In the present work, a study is made of change in the size of the virions when the virus is acted upon by a mutagenic factor, 5-fluorouracil, and the relationship between the sizes of the virions and some genetic markers of the VEE virus is analyzed.

Materials and methods. The method for determining the size of virions has

been described earlier. Only one parameter is presented to characterize the virion size, namely the value of the large axis of the virus particle. The relationship between the size of the virions and various genetic markers were studied in clones isolated from the natural virus population (No. 5, 6, 8, 3/5, 15, 1-1, 12-7, 12-8), and in temperature mutants obtained by us (clones No. 7 and 14).

Formulas and tables from biometry textbooks by Beyli (Bailey?) /1/ and V. Yu. Urbakh /2/ were used in finding correlation coefficients (r) between the size of the virions and various genetic markers.

Change of the size of clones in the course of an induced mutation process was studied in experiments with large-plaque clone 3/5, isolated from a natural virus population. 5-fluorouracil was used as the mutagenic factor. In preliminary experiments it was established that when acting upon a virus that is reproducing in chick fibroblasts, 5-fluorouracil induces the formation of medium-plaque mutants, which differ among themselves with respect to plaque size and with respect to sensitivity to the inhibiting action of agar polysaccharides. The relative share of mutants in a virus yield for one individual development cycle of the virus reaches 30%. The mutated virus retains genetic stability during deactivation in chick fibroblast cultures with the use of conventional nutritive media without the addition of a mutagen. Taking these data into account, we obtained mutants by infecting the cultures with small doses of virus: 30-50 $TTsD_{50}$ per test-tube culture. The virus yield was collected 18 hours after infection. With the experiment set up in this manner, the virus was subjected to the mutagen for several generations, and mutants accumulated in the cultures. Special experiments showed that the content of mutants in the culture liquid attained 99.5%. The obtained virus was subjected to study in an electron microscope.

The genetic characteristics of the VEE virus and their phenotypic manifestation well expressed in the following indicators: S - plaque size in a chick fibroblast culture. The mean diameter (in mm) is given; P_{60} - resistence of the culture to warming at 60°. Indexes of inactivation in lg CBU per 0.2 ml after warming for 10 minutes are presented. The initial virus suspensions contained the virus in a quantity of 10^7 - $10^{7.5}$ CBU per 0.2 ml. i/c - pathogenicity for strainless white mice weighing 8-9 g with intracerebral administration. The virus titer is LD_{50} per 0.03 ml. The titer of the tested virus with titration by the plaque method is 10^7 - $10^{7.5}$ CBU per 0.2 ml. ia - sensitivity to inhibiting action of agar polysaccharides. (-) - resistent clones, the quantity and size of plaques under an agar coating which includes 0.6 mg/ml protamine sulfate, does not increase. (+) - sensitive clones, corresponding indicators increase. AInt - capacity of the virus for autoinfection in a culture of chick fibroblasts. (+) can, (-) cannot. rct_{40} - capacity of virus for multiplication at 40°. (+) virus forms plaques at 40°, (-) does not.

Results and discussion. Data for judging the relationship between the size of the virus particles and the biological properties of the VEE virus are presented in Table 1. For clones isolated from a natural virus population, the presence of a very close inverse correlation is noted between the size of the virions and the size of the plaques; this leads to consideration of the possibility of a relationship between the indicated properties. Apparently the size of the virions affects the size of the plaques, since particles of different size have a different rate of diffusion in agar. By virtue of this, clones with small virions form larger plaques than do clones with large virions.

Table 1. Correlation between the size of virions and some genetic characteristics of the VEE virus.

(a) № клона	(b) Происхождение	(e) Величина вириона (в ммк)	(f) Фенотипическое проявление генетических признаков					
			S	T_{40}	i/c	Ia	AInt	rct_{40}
15	(c)	72,68	9,8	1,4	8,1	+	+	+
3/5		73,11	9,5	1,0	7,6	+	+	+
1—1	Изолированы из	75,20	7,2	5,5	8,5	+	+	+
3	естественной ви-	79,23	9,7	1,1	7,8	+	+	+
12—7	русной популя-	81,03	4,2	3,3	6,3	+	—	+
12—8	ции	82,00	3,9	4,6	5,8	—	+	+
5		82,18	3,2	5,4	7,2	—	—	+
6		86,12	3,4	1,2	7,5	+	—	+
			$r=-0,95$	$r=0,21$	$r=-0,55$			
7	(d) Температурные му-	78,76	1,4	2,5	0,5	—	—	—
14	танты	71,03	0,2	6,2	0,5	+	—	—

Note. Here and in Table 2: designations of genetic characteristics, see in the text.

Key to Table 1: a) clone number; b) origin; c) isolated from a natural virus population; d) temperature mutants; e) size of virions (in millimicrons); f) phenotypic manifestation of genetic characteristics

As far as thermostability is concerned, this property of the virus is not determined by the sizes of the virus particles, since there is no correlation between these indicators. All the studied clones, independently of the sizes of the virions, possessed considerable pathogenicity for strainless white mice with infection via brain, and possessed a capacity for multiplication at 40°. Clones with

relatively small and middle-sized virions (classification of clones in relation to size of the virions was presented earlier) are characterized by a combination of capability for autoinfection in chick-culture fibroblasts, with sensitivity to the inhibiting action of agar polysaccharides. In clones with large virions, these genetic characteristics had a different phenotypic manifestation.

The regularities noted above did not extend to temperature mutants of the VEE virus. The studied mutants formed small or very small plaques, although they were characterized by virions of small size. Apparently the plaque size in these deeply changed mutants, which do not possess the capacity for multiplication at 40° nor pathogenicity for white mice, is determined not by the diffusion rate of virus particles in agar, but by some other factor.

Table 2. Change of genetic markers of the VEE virus in the process of mutations induced by 5-fluorouracil

Вирус	Величина вирионов: размер большой оси $\bar{X} \pm S$ (в ммк)	коэффициент вариации (%)	Фенотипическое проявление генетических признаков: S	Ia	T_{40}
Исходный клон 3/5	73,11±4,92	6,7	9,5	+	1,0
Мутировавший	80,68±7,80	9,6	Неоднородные 2,0—5,0	+ и —	6,4
Субклоны 1 2 3 4 6 7	80,71±5,17 78,70±6,27	6,4 7,9	4,1 3,8 4,7 2,2 2,7 3,5 4,6	— — — — + + +	6,3 6,8

characteristic. The mutated virus is characterized by larger virions than the parent virus, and constitutes a population that is heterogeneous with respect to the sizes of the virus particles. Thus, the particle size is among the characteristics that are subjected to changes in the course of the mutation process.

It is interesting that mutants induced by 5-fluorouracil, which in distinction from the initial virus are characterized by large virions, lose the capacity to form large plaques. This fact makes it possible to think that the correlation described above between the size of the virions and the dimension of the plaques is of a causal nature.

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